

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 5, line 35, through page 6, lines 1-5, with the following amended paragraph:

In another embodiment, the test compound is selected from the ~~th~~ group comprised of: a T-bet nucleic acid molecule, a T-bet peptide, a small molecule T-bet agonist and a small molecule T-bet antagonist. In yet another embodiment, the test compound is selected from a group comprised of: an intracellular antibody, a nucleic acid molecule that is antisense to a T-bet molecule, a dominant negative T-bet molecule, a small molecule T-bet agonist and a small molecule T-bet antagonist. In another embodiment, the cell is selected from the group consisting of: a T cell, a B cell, and a macrophage. In a particularly preferred embodiment, the cell is a Th1 cell.

Please replace the paragraph beginning at page 6, lines 22-27, with the following amended paragraph:

Figures 1A-1B show ~~Figure 1A shows~~ a nucleotide sequence alignment of murine and human T-bet. The alignment was prepared using the ALIGN program. Figures 1C-1F show ~~Figure 1B shows~~ an amino acid sequence alignment of murine and human T-bet prepared using the Lipman Pearson protein alignment program. The T-box sequence is shown in bold. Tyrosine phosphorylation sites are underlined. The nuclear localization site is marked with arrows.

Please replace the paragraph beginning at page 6, lines 6-13, with the following amended paragraph:

Another aspect of the invention pertains to a method of diagnosing a subject for a disorder associated with aberrant immune cell activation comprising:

detecting expression of T-bet in immune cells of a subject suspected of having said disorder;

comparing expression of T-bet in immune cells of said ~~said~~ subject to a control that is not associated with aberrant immune cell activation; and

diagnosing the subject for a disorder based on a change in expression of T-bet in immune cells of the subject as compared to the control.

Please replace the paragraph beginning at page 6, lines 32-35, with the following amended paragraph:

Figure 3A shows that T-bet is preferentially ~~preferentially~~ expressed in double negative thymocytes. Panel B shows that in a survey of Th clones, T-bet expression is restricted to Th1 cells. Panel C shows western blot analysis of T-bet. Panel D shows FACS analysis of T-bet expression.

Please replace the paragraph beginning at page 9, lines 12-27, with the following amended paragraph:

Computer algorithms known in the art can be used to optimally align and compare two nucleotide or amino acid sequences to define the percent identity between the two sequences. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-68, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, *et al.* (1990) J. Mol. Biol. 215:403-10. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, (1997) Nucleic Acids Research 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used. ~~See <http://www.ncbi.nlm.nih.gov>.~~ For example, the nucleotide sequences of the invention were blasted using the default Blastn matrix 1-3 with gap penalties set at: existence 5 and extension 2. The amino acid sequences of the invention were blasted using the default settings: the Blosum62 matrix with gap penalties set at existence 11 and extension 1.

Please replace the paragraph beginning at page 31, lines 21-30, with the following amended paragraph:

An isolated T-bet protein, or fragment thereof, can be used as an immunogen to generate antibodies that bind specifically to T-bet using standard techniques for polyclonal and monoclonal antibody preparation. The T-bet protein can be used to generate antibodies. For example, polyclonal antisera, can be produced in rabbits using full-length recombinant bacterially produced T-bet as the immunogen. This same immunogen can be used to produce mAb by immunizing mice and removing spleen cells from the immunized mice. Spleen cells from mice mounting an immune response to T-bet can be fused to myeloma cells, *e.g.*, SP2/O-Ag14 ~~myeloma myeloma~~. As described in the appended examples, this methods were used to make polyclonal and monoclonal antibodies which bind to T-bet.

Please replace the paragraph beginning at page 55, lines 14-31, with the following amended paragraph:

D. *Autoimmune Diseases*

The inhibitory methods of the invention can be used therapeutically in the treatment of autoimmune diseases that are associated with a Th2-type dysfunction. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and that promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Modulation of T helper-type responses can have an effect on the course of the autoimmune disease. For example, in experimental allergic encephalomyelitis (EAE), stimulation of a Th2-type response by administration of IL-4 at the time of the induction of the disease diminishes the intensity of the autoimmune disease (Paul, W.E., *et al.* (1994) *Cell* 76:241-251). Furthermore, recovery of the animals from the disease has been shown to be associated with an increase in a Th2-type response as evidenced by an increase of Th2-specific cytokines (Koury, S. J., *et al.* (1992) *J. Exp. Med.* 176:1355-1364). Moreover, T cells that can suppress EAE secrete Th2-specific cytokines (Chen, C., *et al.* (1994) *Immunity* 1:147-154). Since stimulation of a Th2-type response in EAE has a protective effect against the disease, stimulation of a Th2 response in subjects with multiple sclerosis (for which EAE is a model) is likely to be beneficial therapeutically. The inhibitory methods of the invention can be used to

affect ~~effect~~ such a decrease.

Please replace the paragraph beginning at page 56, line 35, through page 57, lines 1-14, with the following amended paragraph:

Non-limiting examples of autoimmune diseases and disorders having an autoimmune component that may be treated according to the invention include diabetes mellitus, arthritis (including rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis), multiple sclerosis, myasthenia gravis, systemic lupus erythematosus ~~erythematosus~~, autoimmune thyroiditis, dermatitis (including atopic dermatitis and eczematous dermatitis), psoriasis, Sjögren's Syndrome, including keratoconjunctivitis sicca secondary to Sjögren's Syndrome, alopecia areata, allergic responses due to arthropod bite reactions, Crohn's disease, aphthous ulcer, iritis, conjunctivitis, keratoconjunctivitis, ulcerative colitis, asthma, allergic asthma, cutaneous lupus erythematosus, scleroderma, vaginitis, proctitis, drug eruptions, leprosy reversal reactions, erythema nodosum leprosum, autoimmune uveitis, allergic encephalomyelitis, acute necrotizing hemorrhagic encephalopathy, idiopathic bilateral progressive sensorineural hearing loss, aplastic anemia, pure red cell anemia, idiopathic thrombocytopenia, polychondritis, Wegener's granulomatosis, chronic active hepatitis, Stevens-Johnson syndrome, idiopathic sprue, lichen planus, Crohn's disease, Graves ophthalmopathy, sarcoidosis, primary biliary cirrhosis, uveitis posterior, and interstitial lung fibrosis.

Please replace the paragraph beginning at page 62, lines 4-14, with the following amended paragraph:

T cell assays

Naive CD4+ T cells were purified from spleen and lymph nodes by negative selection (R&D Systems, Minneapolis, MN) and stimulated for 48-72 hours in RPMI/10% with 1 µg/mL anti-murine CD28 (37.51) antibody and 1 µg/mL plate-bound anti-murine CD3 (145-2C11) antibody (BD Pharmingen). Cytokine production was evaluated in culture supernatants by ELISA (BD Pharmingen, San Diego, CA). Proliferation was measured by BrdU incorporation (Amersham Pharmacia Biotech, Piscataway, NJ). Apoptosis was evaluated ~~evaluted~~ by exposing the cells for 24 hours to 20 µg/mL soluble anti-mouse CD3 and anti-mouse CD28, 5

µg/mL dexamethasone (Sigma), or 1200J UV irradiation in a Stratalinker (Stratagene, La Jolla, CA), followed by evaluation by the CaspACE™ Assay System (Promega Corporation, Madison, WI).

Please replace the paragraph beginning at page 62, lines 16-26, with the following amended paragraph:

Immunoglobulin assays

For *in vitro* analyses, ~~purified~~ ~~purified~~ mature B cells were isolated from spleen and lymph nodes by magnetic CD43 depletion (Miltenyi Biotec, Auburn, CA) and stimulated in RPMI/10% with 25 µg/mL LPS (Sigma) supplemented with recombinant murine IL-4 at 10 ng/mL, IFN-γ at 100 ng/mL, human TGF-β1 at 1 ng/mL (PeproTech, Rocky Hill, NJ), or murine IFN-γ at 100U/mL (R&D Systems, Minneapolis, MN). For retroviral infection studies, ~~purified~~ ~~purified~~ CD43-depleted mature B cells were stimulated by 25 µg/mL LPS for 24 hours, followed by infection by a T-bet-GFP or control-GFP retrovirus². Quantitation of serum immunoglobulin isotypes in serum or culture supernatants was performed as previously described³⁴. Germline and postswitch transcripts were determined by RT-PCR as described previously³⁵.

Please replace the paragraph beginning at page 64, lines 15-35, with the following amended paragraph:

Example 3. T-bet binds to and transactivates consensus T-box sites and has functionally important domains that map to both 5' and 3' regions

Recombinant T-bet protein binds to consensus T-box sites and to the T-bet site in the IL-2 promoter, and a complex present in nuclear extracts from anti-CD3-stimulated AE7 Th1 cells binds specifically to a consensus (GGGAATTTACACCTAGGTGAAATTCC) SEQ ID NO:5 T-box oligonucleotide probe. To test for activity of T-bet in T cells, the following experiments were performed. Jurkat Th1 cells were ~~cotransfected~~ ~~cotransfected~~ with T-bet and a luciferase reporter construct. Figure 2A shows the basal level (open bars) and the PMA (50ng/ml) plus ionomycin (1µM) induced (closed bars) promoter activity in Jurkat cells of a luciferase reporter construct containing a minimal thymidine kinase (TK) promoter with or without 4 copies of the

consensus T-box site. Each reporter construct was co-transfected with empty pCDNA vector or pCDNA containing the full-length T-bet cDNA as indicated in the figure. The data shown are representative of three independent experiments. Figure 2B shows Jurkat cells transiently transfected with the luciferase reporter construct containing the minimal TK promoter and multimerized consensus T-box sites and pCDNA vector containing the indicated regions of the T-bet cDNA diagrammed at the left of the bar graph. Luciferase activity was measured 24 hours post-transfection. The experiment was repeated three times with similar results. The basal level (open bars) and the PMA (50ng/ml) plus ionomycin (1uM) induced (closed bars) promoter activity obtained demonstrate that T-bet is active in T cells, and that its activity can be further increased upon stimulation.

Please replace the paragraph beginning at page 65, lines 5-20, with the following amended paragraph:

Figure 3A shows that T-bet is preferentially expressed in double negative (DN) thymocytes, not in double positive (DP) or single positive (SP) cells. Northern blot analysis of total cellular RNA isolated from Th1 cell clones (AE7 and D1.1) or Th2 clones (D10 and CDC35) that were treated with media or with plate-bound anti-CD3 (2C11) for 6 hours revealed T-bet transcripts only in the Th1 clones. Total cellular RNA was isolated from Th1 cell clones (AE7 and D1.1) or Th2 clones (D10 and CDC35) that were treated with media or with plate-bound anti-CD3 (2C11) for 6 hours. Total RNA was also isolated from M12 (B-cell lymphoma) and EL4 (T-cell thymoma) treated with media or with PMA (50ng/ml) and ionomycin (1uM) for 6 hours. Northern blot analysis was performed with 10 ug of total RNA per lane using standard procedures and probed using the full-length T-bet cDNA. T-bet is preferentially ~~preferentially~~ expressed in Th1 clones. Further, the level of T-bet expression was augmented by signals transmitted via the TcR as evidenced by the induction of T-bet transcripts by anti-CD3. T-bet transcripts were not detected in M12, a B-cell lymphoma, in the Th1 lymphoma Jurkat or in EL4, a Th0-cell thymoma either when these cells were treated with media or with PMA (50ng/ml) and ionomycin (1uM) for 6 hours.

Please replace the paragraph beginning at page 65, line 21, through page 66, lines 1-5, with the following amended paragraph:

To determine protein levels of T-bet in primary T cells, DO11.10 TcR transgenic splenocytes were cultured under Th1 or Th2 polarizing conditions. At 72 hours the cells were expanded 3-fold in fresh medium with 200U/ml IL-2. On day 7 after primary stimulation, nuclear and cytosolic extracts were prepared from resting or PMA/ionomycin activated (1 hr) bulk culture DO11.10 Th1 and Th2 cells. Nuclear extracts were also prepared from resting M12, EL4, Jurkat, NK3.3, and YT cells. As shown in Figure 3C, among the cell lines, T-bet protein was present in YT cells only. Figure 3C shows T-bet protein is restricted to Th1 cells and NK cells. Western blot analysis was performed on nuclear and cytosolic extracts prepared from resting or PMA/ionomycin activated (1 hr) bulk culture DO11.10 Th1 and Th2 cells as above. Briefly, DO11.10 TcR transgenic ~~TcR transgenic~~ splenocytes were activated with OVA peptide (323-339) at 3×10^6 cells/ml in the presence of 10 ng/ml IL-12 and 10 ug/ml anti-IL-4 (11B11) to promote Th1 phenotype development, or 10ng/ml IL-4 and 10 ug/ml anti-IFN-gamma to promote Th2 phenotype development. At 72 hours the cells were expanded 3-fold in fresh medium with 200 U/ml IL-2. On day 7 after primary stimulation, nuclear and cytosolic extracts were prepared from resting or PMA/ionomycin activate ~~activate~~ (1hr) bulk culture DO11.10 Th1 and Th2 cells. Nuclear extracts were also prepared from resting M12 cells, EL4, Jurkat, NK3.3, and YT. 30 ug of nuclear and cytosolic extracts were separated by SDS-PAGE (8% gel), transferred to nitrocellulose, and probed with an anti T-bet antisera. In primary T cells, T-bet protein is selectively expressed in T cells driven along a Th1 but not a Th2 pathway, consistent with the Northern blot analysis of T cell clones and primary T cells shown above.

Please replace the paragraph beginning at page 67, line 36, through page 68, lines 1-3, with the following amended paragraph:

The activity of a luciferase reporter construct containing 9 kb of the IFN-gamma gene in the Jurkat human Th1 lymphoma and the mouse EL4 Th0 thymoma ~~thymoma~~ was tested. Each reporter construct (10ug) was co-transfected with empty pCDNA vector or pCDNA containing the full-length T-bet cDNA, c-Maf, NFATp or p65 (10ug). The constructs also include the -400 to -40 IL-2 and IL-4 promoter luciferase reporters.

Please replace the paragraph beginning at page 68, lines 30-37, with the following amended paragraph:

A bovine collagen-specific Th0 hybrid was transduced with retroviral constructs containing T-bet GFP or GFP only under the control of the TcR inducible IL-2 promoter. Transduced populations were FACS sorted on GFP twice, rested and then stimulated with anti-CD3 and supernatants ~~supernatants~~ collected at 60 hours to measure cytokine production by ELISA. (Figure 6). Control retroviral vectors which had not affect ~~effect~~ included anti-sense T-bet.

Please replace the paragraph beginning at page 69, lines 7-15, with the following amended paragraph:

BALB/c CD4 T cells were infected after 36 hours of primary activation ~~activation~~ by anti-CD3 plus anti-CD28, harvested on day 7 and intracellular IFN-gamma and IL-2 staining performed 5 hours after stimulation with PMA and ionomycin as described in Experimental Procedures. Data are shown ~~shown~~ as two-color plots showing GFP expression (FL1) versus intracellular cytokine (FL2) of events gated on expression of CD4. Primary T cells from MBP TcR transgenic mice were stimulated using MBP (Ac1-11) at 6 uM and infection performed on day 1 with IL-2/GFP and IL-2/T-bet/GFP. On day 7, cells were sorted for GFP expression, rested for 1 day and then intracellular cytokine analysis performed after a 5 hour stimulation with PMA and ionomycin.

Please replace the paragraph beginning at page 71, lines 1-10, with the following amended paragraph:

Example 10. T-bet also redirects polarized Tc2 [cells] ~~ells~~ into the Th1 pathway

Although most attention has focused on the CD4⁺ T lymphocyte, it is apparent that cytotoxic CD8⁺ T cells also may also be divided into IFN- γ -producing (Tc1) and IL-4-producing (Tc2) subsets. The ability of T-bet to redirect fully polarized Tc2 cells into a Tc1 pathway was tested. Purified CD8⁺ T cells were therefore ~~therefore~~ differentiated in culture under Tc2 polarizing conditions for 9 days to accomplish full differentiation. Figure 10 .

demonstrates that T-bet transduced Tc2 cells, similar to T-bet transduced CD4 Th2 cells have been reprogrammed to produce IFN- γ (85% versus 15%) and to repress the production of IL-4 and IL-5 (3% versus 34% and 1% versus 45% respectively). Thus, T-bet can convert fully differentiated CD8+ Tc2 cells to Tc1 cells.

Please replace the paragraph beginning at page 71 line 33, through page 72, lines 1-13, with the following amended paragraph:

Example 13. Mutations of the T box of the IL-2 promoter decrease IL-2 promoter activity

Recently, the crystal structure of the T-box region of the Brachyury gene bound to DNA was solved and the amino acid moieties essential for specific DNA contacts or for minor contacts deduced. Examination of the human and murine IL-2 proximal promoter shows that the critical nucleotides for binding a T-box family member are present. Specifically -240 to -220 bp of the murine IL-2 promoter has strong similarity to the consensus T-box site. The consensus T-box site is AATTTCACACCTAGGTGTGAAATT (SEQ ID NO:6). The human IL-2 promoter comprises: gAgcTatCACCTAaGTGTGggcTa (SEQ ID NO:7). The murine IL-2 promoter comprises: AAacTgcCACCTAaGTGTGggcTa (SEQ ID NO:8). The mutated T-box mIL-2 promoter comprises: AAacTgctgtCTAaacaTGggcTa (SEQ ID NO:9). (DNA contacts are in bold, minor contacts are underlined). Transversional nucleotide substitutions shown by crystal structure to be essential for DNA-protein interactions were made within this putative T-box site in the context of the murine -440 to -40 bp IL-2 promoter. The basal level (open bars) and the PMA (50ng/ml) plus ionomycin (1uM) induced (closed bars) promoter activity in Jurkat cells (left) or AE7 Th1 clone (right) of IL-2 luciferase reporter constructs is shown (Figure 13).

Please replace the paragraph beginning at page 75, line 26, through page 76, lines 1-4, with the following amended paragraph:

Example 15. T-bet is Required for Interferon- γ Production and Lineage Commitment in CD4 but not CD8 T Cells

T-bet is expressed in both CD4 and CD8 T cells. To determine whether T-bet is involved with IFN- γ production in both CD4 and CD8 T cells, the following experiments were

~~performed~~ performed. Purified CD4 and CD8 T cells were stimulated for 72 hrs with plate bound anti-CD3, anti-CD28, rIL-12 and rIL-18, RNA prepared and northern blot analysis performed using T-bet, IFN- γ , and HPRT probes. CD8 T cells and CD4 T cells purified from T-bet^{-/-}, T-bet^{+/-} and T-bet^{+/+} LN were stimulated with plate-bound anti-CD3 and anti-CD28 for 7 days. ICC analysis was performed after 5 hours stimulation with PMA (50ng/ml) and ionomycin (1uM). IFN- γ production was measured by ELISA 24 hrs after restimulation with anti-CD3/anti-CD28. CTL precursors from T-bet^{+/+} or ^{-/-} splenocytes were primed *in vitro* with Concanavalin A (5 ug/ml or plate bound anti-CD3/anti-CD28 and 100 U/ml hIL-2 for 5 days (32). On day 5 CD8 T cells (H-2^b) were purified by positive selection using MACS purification and incubated for 4 hours with ⁵¹Cr labeled P815 (H-2^d) allogeneic target cells at the indicated effector to target ratios.

Please replace the paragraph beginning at page 81, lines 3-11, with the following amended paragraph:

To determine the susceptibility of mice in which the T-bet gene has been inactivated by homologous ~~recombination for recombination~~ for T cell-mediated colitis, T-bet deficient mice that exhibited an altered susceptibility to Th2-mediated colitis using the oxazolone-induced colitis model that has previously been shown to be dependent on IL-4 production by T cells were ~~analyzed~~ ~~analyzed~~. The T-bet knockout mice showed enhanced susceptibility to oxazolone-induced colitis compared to both wild-type littermates and heterozygous T-bet mice, based on (by weight curve), macroscopic and histopathologic criteria. This was accompanied by a marked increase in IL-4 production by splenic CD3⁺ T cells, while IFN- γ production by these cells was not significantly changed.

Please replace the paragraph beginning at page 83, line 28, through page 84, lines 1-18, with the following amended paragraph:

Regulatory T cells producing IL-10 or TGF- β mediate protective effects in Th1-mediated colitis by suppressing the activity of T lymphocytes. To determine if T-bet has a role in TGF- β production and signaling in regulatory T cells, the following studies were performed. Cells were cultured in the presence of antibodies to CD3 and CD28 with or without recombinant

IL-4 and TGF- β (1 ng/ml). Cellular extracts were made after 48 hours and analyzed for the expression of T-bet and beta-actin by Western blot analysis. To determine whether TGF- β is produced by ~~by~~ T cell enriched LPMC from wild-type (WT), T-bet heterozygous (HET) and T-bet knockout (KO) mice in the absence of colitogenic stimuli, cells were stimulated with antibodies to CD3 plus CD28 and supernatants were analyzed by ELISA. To determine whether T-bet is expressed in splenic CD25⁺, CD62L⁺ and CD62L⁻ CD4⁺ T cells from healthy wild-type mice, cytoplasmic (CYT) and nuclear (NUC) extracts from these cells were isolated and analyzed for T-bet expression by Western blotting. To determine whether TGF- β -mediated signaling is increased in T-bet deficient CD62L⁻ CD4⁺ T cells, CD62L⁻ CD4⁺ T cells from wild-type and T-bet knockout mice were stimulated with anti-CD3 plus anti-CD28 and rIFN- γ for 12 hours followed by protein extraction and Western blot analysis. Cellular extracts were analyzed for Smad7 expression whereas nuclear extracts were analyzed for Smad3 levels. To measure the inflammation score of mice reconstituted with CD62L⁺ CD4⁺ T cells from wild-type mice and CD62L⁻ CD4⁺ T cells from T-bet knockout mice (KO) and wild-type (WT) control mice were measured.

Please replace the paragraph beginning at page 84, lines 19-36, with the following amended paragraph:

The foregoing studies demonstrate a regulatory role for T-bet in mucosal cytokine production. Specifically, the present invention demonstrates that CD62L⁻ CD4⁺ T cells from T-bet knockout mice exhibit a stronger protective effect on CD62L⁺ CD4⁺ T cell-induced colitis than the corresponding cell population from wild-type mice. This observation is related to differences in TGF- β production and signaling, as CD62L⁻ CD4⁺ T cells from T-bet deficient mice exhibited increased nuclear Smad3 expression. After binding of TGF- β to its receptor on T cells, Smad3 is interacts with the TGF- β receptor I followed by importin-1 β and RanGTPase-mediated import of Smad3 into the nucleus where it controls expression of β target genes. IFN- γ has been previously shown to inhibit TGF- β signaling by a Jak1/STAT-1-mediated rapid activation of the synthesis of the inhibitory Smad-7 protein, which in turn can prevent the

interaction of Smad3 with the TGF- β type I receptor. Furthermore, Smad7 can form a complex with the ubiquitin-ligase Smurf2 that targets the TGF- β receptor for degradation. Thus, the reduced production of IFN- γ by splenic CD4⁺ T cells and T cell enriched lamina propria cells in T-bet deficient animals causes ~~causes~~ reduced expression of Smad7 followed by increased TGF- β signaling via Smad3/4. In fact, CD62L⁻ CD4⁺ T cells from T-bet deficient mice express reduced levels of Smad7 compared to T cells from wild-type mice.

Please replace the paragraph beginning at page 86, lines 2-19, with the following amended paragraph:

Naïve mice, *i.e.*, neither antigen sensitized nor challenged, with a targeted deletion of T-bet were examined to ascertain if such animals would manifest various aspects of the induced asthma phenotype. Compared to wild type (wt) mice, those either heterozygous (T-bet +/-) or homozygous for a targeted deletion of T-bet (T-bet -/-) exhibited greater airway responsiveness, as measured in unanesthetized animals by the enhanced pause response (Penh), following aerosol exposure to methacholine. These findings were confirmed in mice that had been sensitized by systemic exposure to ovalbumin but sham challenged with aerosol phosphate buffered saline (termed OVA/PBS), by the demonstration that both T-bet (+/-) and (-/-) mice manifested airway hyper-responsiveness, as compared to wt mice, when the pulmonary resistance response resulting from intravenous infusion of methacholine, was used as the outcome indicator. Histopathologic analysis of the airways of T-bet (-/-) mice at baseline demonstrated peribronchial and perivenular infiltration with eosinophils and lymphocytes as compared to control wild-type wt-littermates ~~littermates~~. T-bet deficient mice had increased deposition of fibroblast-like cells beneath the basement membrane. Eosinophils were not present in the bronchoalveolar lavage fluid of T-bet deficient mice despite enhanced recovery of IL-5. T-bet +/- heterozygous mice, that have only a 50% reduction in T-bet protein, displayed a phenotype very similar to mice with a complete absence of T-bet.

Please replace the paragraph beginning at page 87, line 16, through page 88, line 1, with the following amended paragraph:

The identity of the cells in T-bet deficient mice responsible for airway hyperreactivity and airway inflammation was examined by adoptive transfer of spleen CD4⁺ cells from different groups of OVA sensitized mice into histocompatible SCID mice. To enhance the localization of the transferred T cells into the lungs of mice, the OVA aerosol was administered one day before adoptive transfer of the T cells. On the day following the adoptive transfer, OVA aerosol exposures were begun and continued for three days. Four days after cell transfer, lung mechanics were evaluated. Control SCID mice received an intraperitoneal infusion of saline rather than T cells suspended in saline. Recipients of wt spleen CD4 cells had comparable airway responsiveness to wt mice that received OVA sensitization but were not challenged. Mice that had been reconstituted with CD4 cells lacking T-bet showed increased airway hyperresponsiveness as compared to mice reconstituted with CD4 cells derived from wild-type wt-littermates ~~littermates~~ and similar to that of OVA sensitized mice lacking T-bet. CD4 staining of BALF cells harvested after measurement of lung mechanics was performed to assure that CD4⁺ cells were recruited to the lung; the proportion of lymphocytes that were CD4 positive in wt (+/+) mice was 38.9% +/- 2.2; in CD4 T-bet (+/-) mice was 39.57% +/- 6.48; and in T-bet (-/-) mice was 38.5% +/- 5.48. In addition, the lungs of SCID mice reconstituted with CD4 cells derived from T-bet (-/-) mice exhibited increased IL-4 in the BALF as compared to recipient mice reconstituted with spleen CD4⁺ cells derived from wt mice, demonstrating that the airway hyperactivity observed in T-bet (-/-) mice is T-cell mediated.